

# Islands in the stream: Distribution of *Myotis attenboroughi* (Chiroptera, Vespertilionidae) in Grenada and mainland South America illuminates the evolutionary history of Caribbean *Myotis*

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## Abstract

*Myotis attenboroughi* was recently described based on specimens from Tobago Island and considered an endemic species until now. Based on morphological and molecular data, we extended its occurrence to Grenada and Suriname. The presence of *M. attenboroughi* in Grenada, Tobago and mainland South America can be explained by the intermittent connections between these islands and the continent during the Plio-Pleistocene glaciations. However, our analyses recovered an *Myotis* individual from Grenada phylogenetically related to *Myotis nyctor* from Barbados, although it is morphologically like the other specimens of *M. attenboroughi*, revealing a surprising incongruence between genotype and phenotype. Barbados is a geologically recent island and historically unconnected with other Caribbean islands or with South America. We hypothesized that the invasion of the *M. nyctor* lineage from Barbados to Grenada was the result of an extreme overwater dispersal, perhaps driven by wind streams during tropical storms. Subsequently, introgression of lineages may have occurred through hybridization, which can explain the discordance between the phenotype (like *M. attenboroughi*) and the genotype (like *M. nyctor*) of this specimen. Additional comments on the formation of the Caribbean *Myotis* assemblage are presented from the perspective of new evolutionary discoveries for this genus.

## Key Words

Barbados, Caribbean biogeography, historical DNA, *Myotis nyctor*, Suriname, taxonomy

## Introduction

*Myotis* has a worldwide distribution, occurring in every region of the globe except for parts of Australasia and the polar regions and is the most diverse genus within the order Chiroptera, comprising 139 valid species (Moratelli et al. 2019; Simmons and Cirranello 2024). Thirty-six neotropical species are currently recognized (Novaes et al. 2024), seven of which are found on the Caribbean islands (Novaes et al. 2021), being: *M. nesopolus* Miller, 1900

in Bonaire and Curaçao; *M. dominicensis* Miller, 1902 in Dominica and Guadeloupe; *M. martiniquensis* LaVal, 1973 in Martinique; *M. nyctor* LaVal & Schwartz, 1974 in Barbados and Grenada; *M. pilosatibialis* LaVal, 1973 (possibly *M. armiensis* Carrión-Bonilla & Cook, 2021), *M. riparius* Handley, 1960 in Trinidad; and *M. attenboroughi* Moratelli et al., 2017 in Tobago (LaVal 1973; Larsen et al. 2012; Moratelli et al. 2017; Novaes et al. 2021).

Miller and Allen (1928) recognized only *M. nigricans* (Schinz, 1821), with three subspecies in the Caribbean

islands. Populations from Grenada and Trinidad and Tobago were assigned to the nominative subspecies, those from Dominica to *M. nigricans dominicensis* Miller, 1902; and those from Curaçao to *M. nigricans nesopolus* Miller, 1900. Later, LaVal (1973) elevated *dominicensis* to the species level and described *M. martiniquensis* from Martinique and Barbados. LaVal and Schwartz (1974) subsequently recognized Barbados' populations as distinct and described a new subspecies, *M. martiniquensis nyctor*. Genoways and Williams (1979) elevated *nesopolus* to the species level and reported its occurrence in Bonaire, while Genoways et al. (1998) reported *M. nigricans* from Grenada. Masson and Breuil (1992) reported the occurrence of one *Myotis* species from Guadeloupe, presumably assigned to *dominicensis*. Larsen et al. (2012) raised *M. martiniquensis nyctor* to the species level, noting its presence in Barbados, with pending records from Grenada based on three museum specimens that were not examined by the authors. Moratelli et al. (2017) examined these specimens, assigning USNM 254717 to *Peropteryx* (Emballonuridae), and CM 83427 and USNM 252600 to *M. nyctor*. In addition, these authors revised the population of *Myotis nigricans* from Tobago and, based on morphological and molecular evidence, assigned this population to a new species, *Myotis attenboroughi* (see Moratelli et al. 2017). Recently, Novaes et al. (2021) reviewed the Venezuelan and Caribbean *Myotis*, suggesting that populations from Barbados and Grenada may represent distinct taxa, and emphasized the need for additional morphological and molecular data to support this hypothesis.

Based on novel molecular and morphological evidence, we extend the occurrence of *M. attenboroughi* to Grenada (based on the reexamination of the specimen USNM 252600, collected in 1938 and previously assigned to *M. nyctor*) and to mainland South America (based on the specimen CM 77705 previously assigned to *Myotis* cf. *nigricans*). Our reassessment of the taxonomic identity of these and other historical specimens from Tobago (i.e., USNM 540692) and Grenada (i.e., CM 83427) also allowed us to further discuss the evolution of *Myotis* in the Caribbean.

## Material and methods

Our research group conducted a comprehensive review of neotropical *Myotis* by analyzing over 7,500 specimens and hundreds of DNA sequences. This extensive analysis resulted in the recognition of 11 new species and several other nomenclatural acts (e.g., Moratelli et al. 2011, 2013; Novaes et al. 2022a, b). The present study was based on the analysis of datasets comprising molecular, morphometric, and discrete morphological characters. We adopted the Phylogenetic Species Concept (Wheeler 1999), considering monophyly and diagnosability as criteria for species recognition (Gutiérrez and Garbino 2018).

## Molecular data and analyses

Molecular analyses were based on 122 sequences of the mitochondrial cytochrome b gene (cytb, ca. 1,140 bp) from New World *Myotis* species and three outgroups (Appendix 1). Most sequences (123 out of 125, including outgroups) were obtained from NCBI's GenBank, including the sequences from CM 77705 (Suriname) and CM 83427 (Grenada). The remaining two sequences were generated in this study. Tissue samples of *Myotis* from Grenada (USNM 252600) and Tobago (USNM 540692) were obtained from toe clips from historical specimens deposited in the Smithsonian National Museum of Natural History, Washington D.C., USA, following rigorous sampling procedures described in Abreu et al. (2020). DNA extractions were performed in an isolated historical DNA facility at the Smithsonian Center for Conservation Genomics (CCG), using a standard phenol-chloroform protocol (McDonough et al. 2018), including a long (48–72 hours) lysis step. We did not perform specific amplifications for the cytb gene. Fragments of mtDNA were obtained as a byproduct (off-target sequences) of the capture and enrichment of Ultraconserved Elements sequenced for a parallel study conducted by our team. Sequencing was performed on Illumina Hi-Seq 4000 150 PE at the Vincent J. Coates Genomics Sequencing Laboratory at the University of California, Berkeley. A detailed description of the procedures used in the preparation of genomic libraries, DNA quantification, and sequencing is available in Abreu et al. (2020). To obtain the cytb gene sequences, we mapped the clean Illumina reads against a reference mitochondrial genome from GenBank, using the “Map to Reference” tool in Geneious R11 (Kearse et al. 2012). Mapping and sequence assembly parameters followed Abreu et al. (2020).

The cytb dataset was aligned using the UPGMA clustering method implemented in the MUSCLE algorithm (Edgar 2004) in the MEGA X software (Kumar et al. 2018) with default settings. The evolutionary model of nucleotide substitution was chosen for phylogenetic analyses using the software JModelTest 2 (Darriba et al. 2012), employing the Bayesian Information Criterion (BIC). The Hasegawa–Kishino–Yano model (Hasegawa et al. 1985) yielded the best fit to our dataset regarding the substitution of nucleotides, corrected for rate heterogeneity with gamma distribution and proportion of invariant sites parameters (i.e., HKY +  $\Gamma$  + I). Phylogenetic reconstruction was performed using the Bayesian Inference (BI) probabilistic method (Huelsenbeck et al. 2001) in the software MrBayes v. 3.4 (Ronquist and Huelsenbeck 2003) using the coupled Markov Chain Monte Carlo (MCMC). Four simultaneous Markov chains were performed for 100,000,000 generations with trees sampled every 10,000 generations. The first 26,000 trees were discarded as burn-in. Posterior probabilities were calculated from the consensus of the remaining trees. The confidence of the Bayesian sampling was verified for the free parameters using the effective sample size (ESS) statistic implemented in the

software Tracer v. 1.5 (Rambaut and Drummond 2009). Convergence was checked by plotting log-likelihood values against the generation time for each model, with all parameters showing ESS greater than 300 and asymptotically convergence indicating reliable performance.

Pairwise genetic distances within and among *Myotis* species were estimated using the HKY model implemented in ‘ape 5.0’ package for R software (Paradis and Schliep 2019), which measures the distance between pairs of sequences by estimating the proportion of different nucleotides between them.

Morphological data and analyses

For the morphological analyses and comparisons, we examined 14 specimens of *M. attenboroughi* (13 from Tobago, including holotype and paratypes; and one from Grenada); eight of *M. nyctor* from Barbados (including a paratypes); and one specimen from Grenada tentatively identified as *M. cf. nyctor* (Appendix 2). Quantitative morphological data were based on 16 skull dimensions, representing different axes of the length and width of the skull, rostrum, and mandible, and three external measurements (Table 1). Measurements were taken using digital calipers accurate to 0.01 mm, exclusively from individuals classified as adults based on closed epiphyses (see Brunet-Rossini and Wilkinson 2009). Additionally, measurements of total length, tail length, hindfoot length and body weight were recorded from the specimen’s tag and used for comparative purposes only.

Principal Component Analysis (PCA) was used to identify general trends in variation in size and shape vari-

ation of the skull between forms of Barbados, Grenada, and Tobago. This analysis was performed in R using the MASS (Vanables and Ripley 2002) and Lattice packages (Sarkar 2008). As PCA requires a complete dataset without missing data, cranial measurements that could not be taken from the specimen due to skull fractures and losses of parts were estimated from the log-transformed dataset using the EM algorithm implemented in the R package Amelia II (Honaker et al. 2011).

Qualitative morphological analyses were based on six cranial and external characters traditionally used in Neotropical *Myotis* taxonomy (q.v., LaVal 1973; Moratelli et al. 2013; Novaes et al. 2022a). Fur color was also used to describe the variation, following the nomenclature used in the color catalog of Ridgway (1912).

Results

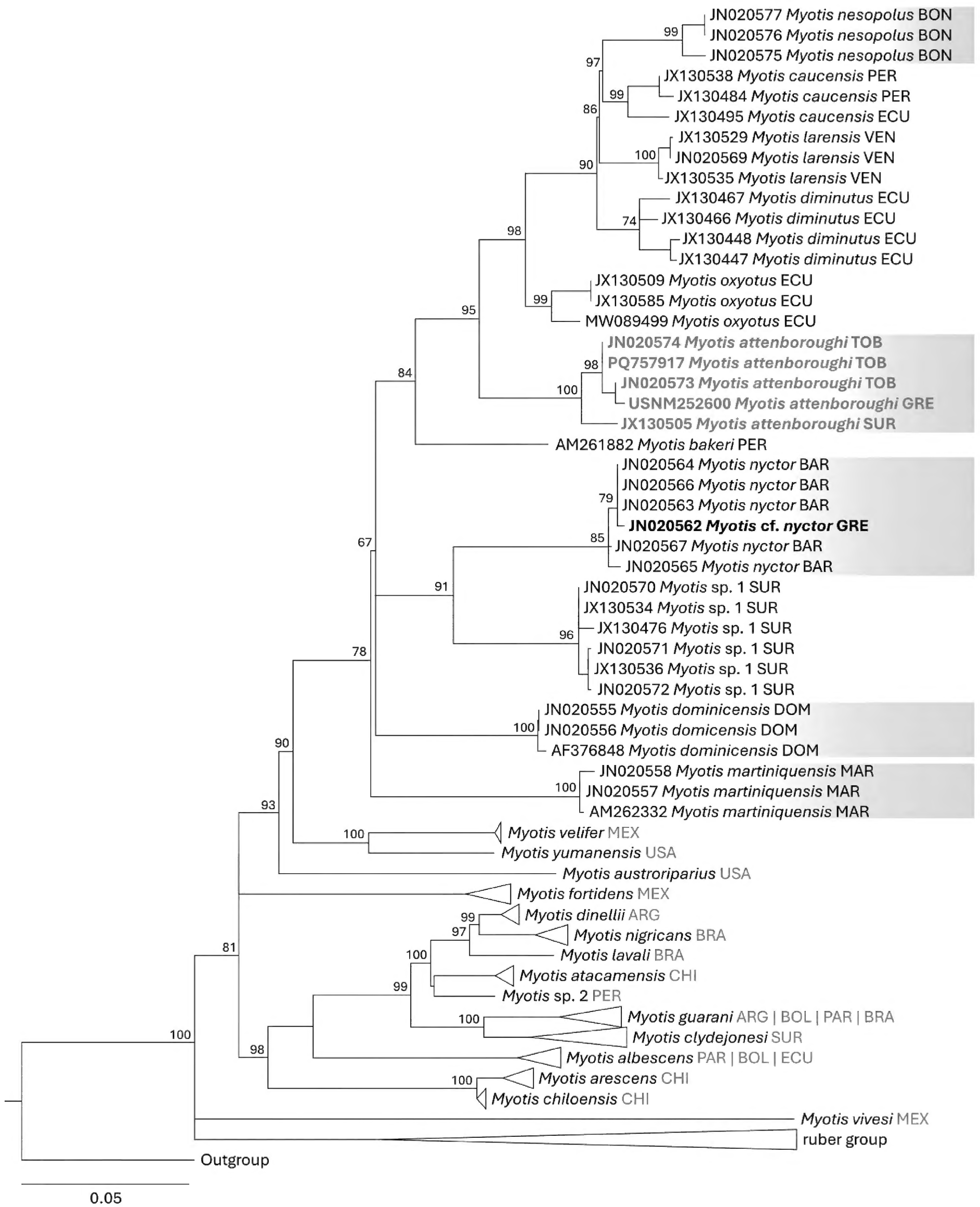
Phylogenetic inference and genetic distances

The specimen of *Myotis* from Grenada (USNM 252600) was recovered within the *M. attenboroughi* clade, which also includes the paratype USNM 540692 (Fig. 1). The specimen from Paramaribo, Suriname (CM 77705), was recovered as sister to all Caribbean samples (Tobago and Grenada) of *M. attenboroughi*. The specimen CM 83427 from Grenada was recovered within a clade composed by specimens of *M. nyctor* from Barbados, and here it is being treated as *M. cf. nyctor*. Both clades corresponding to *M. attenboroughi* and *M. nyctor* are closely related to other *Myotis* species from the Caribbean and northern South America, all included in the *albescens* species group (Fig. 1).

**Table 1.** Skull dimensions used to perform morphological comparisons in Neotropical *Myotis* species. Measurements were taken in millimeters.

| Measurement                        | Description   |
|------------------------------------|---|
| Forearm Length (FL)                | From the elbow to the distal end of the forearm including carpals   |
| Third metacarpal length (3ML)      | From the distal end of the forearm including carpals to the distal end of the 3 <sup>rd</sup> metacarpal. |
| Lenght of dorsal fur (LDF)         | Length of the longest hairs at the midpoint of the scapulae   |
| Length of ventral fur (LVF)        | Length of the longest hairs at the midpoint of the sternum  |
| Mandibular length (MAL)            | From the mandibular symphysis to the condyloid process  |
| Mandibular toothrow length (MAN)   | From the lower canine to third molar  |
| Greatest length of skull (GLS)     | From the apex of upper internal incisors to the occiput   |
| Condylo-canine length (CCL)        | From the anterior surface of upper canines to a line connecting the occipital condyles                    |
| Condylo-incisive length (CIL)      | From the apex of upper internal incisors to a line connecting the occipital condyles                      |
| Condylo-basal length (CBL)         | From the anterior region of premaxilla to a line connecting the occipital condyles                        |
| Basal length (BAL)                 | Least distance from the apex of upper internal incisors to the anterior margin of the foramen magnum      |
| Zygomatic breadth (ZYG)            | Greatest breadth across the outer margins of the zygomatic arches   |
| Mastoid breadth (MAB)              | Greatest breadth across the mastoid region  |
| Braincase breadth (BCB)            | Greatest breadth of the globular part of the braincase  |
| Interorbital breadth (IOB)         | Least breadth between the orbits.   |
| Postorbital constriction (POB)     | Least breadth across frontals posterior to the postorbital bulges   |
| Breadth across canines (BAC)       | Greatest breadth across outer edges of the crowns of upper canines including cingulae                     |
| Breadth across molars (BAM)        | Greatest breadth across outer edges of the crowns of upper molars   |
| Maxillary toothrow length (MTL)    | From the upper canine to third molar  |
| Upper molar toothrow length (M1M3) | From M1 to M3   |





**Figure 1.** Phylogenetic tree based on Bayesian Inference of cytochrome-b sequences of species of Neotropical *Myotis*. Nodal support was calculated by posterior probabilities. Caribbean species are highlighted by gray bars.

The estimated HKY genetic distances indicated that *M. attenboroughi* from Suriname (CM 77705) diverges by approximately 1.5% from samples from Tobago and Grenada. The average divergence within the clade composed of Tobago and Grenada samples of *M. attenboroughi* was less than 0.1%. Genetic distances among *M.*

*attenboroughi* and phylogenetic closely species range from 6 to 8% (Table 2). *Myotis attenboroughi* from Grenada (USNM 252600) diverges by about 10% from *M. nyctor* from Barbados, alike *M. attenboroughi* from Grenada diverges in about 10% from *M. cf. nyctor* from Grenada (CM 83427).

**Table 2.** Average Hasegawa–Kishino–Yano genetic distances within (boldface along diagonal) and among (below diagonal) *Myotis* species based on cytochrome-b gene sequences.

| Species                    | 1              | 2              | 3              | 4              | 5              | 6              | 7              |
|----------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| 1. <i>M. attenboroughi</i> | <b>[0.008]</b> |                |                |                |                |                |                |
| 2. <i>M. larensis</i>      | 0.073          | <b>[0.006]</b> |                |                |                |                |                |
| 3. <i>M. nesopolus</i>     | 0.080          | 0.051          | <b>[0.002]</b> |                |                |                |                |
| 4. <i>M. diminutus</i>     | 0.069          | 0.020          | 0.044          | <b>[0.009]</b> |                |                |                |
| 5. <i>M. caucensis</i>     | 0.090          | 0.041          | 0.040          | 0.037          | <b>[0.031]</b> |                |                |
| 6. <i>M. oxyotus</i>       | 0.059          | 0.063          | 0.072          | 0.044          | 0.069          | <b>[0.015]</b> |                |
| 7. <i>M. nyctor</i>        | 0.111          | 0.132          | 0.145          | 0.116          | 0.137          | 0.124          | <b>[0.006]</b> |

Morphological description and comparisons

The specimen of *M. attenboroughi* from Grenada (USNM 252600) is an adult male preserved as dry skin and skull (Figs 2, 3). It is a small-sized specimen (forearm length 32.6 mm; other measurements in Table 3), with medium-sized ears (14 mm). The fur is silky in texture and medium-sized (LDH 5.6 mm, LVH 4.5 mm). The dorsal fur is bicolor, with dark brown bases (ca. 1/2 of the total length of the fur), and Mummy Brown tips (ca. 1/2), without a defined contrast between the bands. Ventral fur is strongly bicolor, with blackish bases and Light-Buff tips (Fig. 2). Membranes are Mummy Brown. Uropatagium is attached to the foot by a broad band of membrane. Dorsal surfaces of elbow and tibia are naked. The uropatagium lacks the fringe of hairs along the trailing edge.

Like in other neotropical *Myotis*, the dental formula is 2/3, 1/1, 3/3, 3/3 = 38. The skull is comparatively small, lacking a sagittal crest; lambdoidal crests are present, but very low; parietals are slightly inclined (Fig. 3). The occipital region is rounded, projecting behind the posterior surfaces of occipital condyles. The second upper premolar (P3) is aligned in the toothrow, smaller than P2 and P4, and visible in labial view. The set of qualitative morphological characters of the Grenada specimen (USNM 252600) is in accordance with the holotype of *M. attenboroughi* from Tobago (USNM 540693) and with the diagnosis presented in the original description (i.e., Moratelli et al. 2017). The morphometric measurements of this specimen are within the known range for *M. attenboroughi* from Tobago (including holotype and paratypes; Table 3).

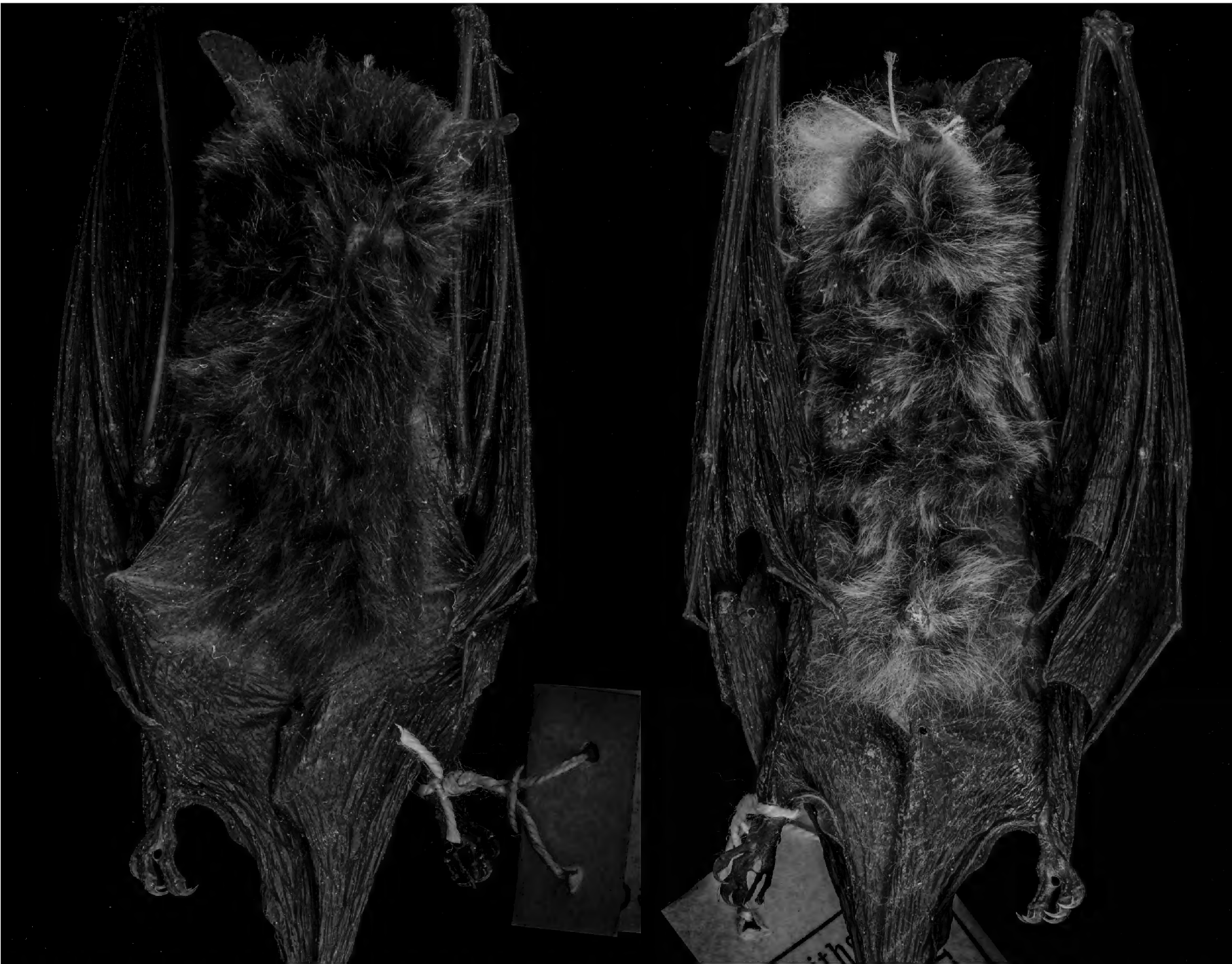
*Myotis attenboroughi* and *M. nyctor* are phenotypically quite similar but they can be distinguished by a set of morphological characters (Figs 4, 5). *Myotis nyctor* has silky, medium-sized fur (LDH 6.3 mm, LVH 4.5 mm). Dorsal fur bicolored, with Cinnamon Brown to Mummy Brown tips (ca. 1/2 of the total length of the fur) and darker bases, without well-marked limits between bands. Ventral fur strongly bicolored, with dark brown bases and Light-Buff tips. *Myotis nyctor* differs from *M. attenboroughi* in general size (Table 3) and craniodental characters, which include its longer and narrow rostrum; larger canines; less globose braincase; and narrower interorbital constriction.

The specimen from Grenada (CM 83427), phylogenetically grouped with *M. nyctor* samples from Barbados,

presented external and skull morphology virtually identical to *M. attenboroughi* specimen (USNM 252600) also from Grenada. The specimen CM 83427 is an adult male preserved as dry skin and skull. Despite exhibiting no discrete distinguishable character, these two individuals from Grenada (CM 83427 and USNM 252600) were also recovered closely positioned within the morphospace based on the PCA analysis. Both specimens presented skull dimensions similar to *M. attenboroughi*, being recovered close to the cluster formed by samples of *M. attenboroughi* from Tobago (Fig. 6). In this analysis, the first principal component (PC1) accounted for almost 98% of the variation, which was mainly driven by the variation found in measurements associated with condylo-incisive length, condylo-basal length, greatest length of skull, and condylo-canine length (Table 4).

Discussion

Our results unequivocally supported the identification of the specimen USNM 252600 from Grenada as *M. attenboroughi*. This specimen was captured by S. Gates in March 1938 and originally identified as *M. nigricans*. Genoways et al. (1998) examined this specimen and collected an additional individual during an expedition to Grenada in June 1986 (CM 83427), confirming their identity as *M. nigricans* and arguing that the specimens from Grenada are very similar to *M. nigricans* from mainland Venezuela. Later, Larsen et al. (2012) sequenced the cytb gene from the specimen CM 83427 and found this specimen within a clade composed of *M. nyctor* from Barbados. However, Larsen et al. (2012) noted that cranial and external measurements of specimens from Grenada were outside the range observed for *M. nyctor* from Barbados, which corroborated the Genoways et al. (1998) observations. Based on qualitative morphological analyses, Moratelli et al. (2017) supported the results of Larsen et al. (2012) and reidentified the specimens USNM 252600 and CM 83427 as *M. nyctor*, thus rejecting the hypothesis of *M. nigricans* occurring in Grenada. In a critical review of Caribbean *Myotis*, Novaes et al. (2021) suggested that the taxonomic status of populations from Grenada still needed to be assessed, considering the presence of morphological disparities and possible retention of ancestral polymorphism resulting from recent speciation.



**Figure 2.** Dorsal (left) and ventral (right) view of the specimen of *Myotis attenboroughi* (USNM 252600) from Grenada Island.

**Table 3.** Selected measurements of *Myotis attenboroughi* and *M. nyctor*. Descriptive statistics include the mean, range (in parentheses), and sample size. Measurements are rounded to the nearest 0.1 mm. See Table 1 for abbreviations.

| Measurements | <i>M. attenboroughi</i> Grenada<br>USNM 252600 | <i>M. attenboroughi</i> Tobago | <i>M. cf. nyctor</i> Grenada<br>CM 83427 | <i>M. nyctor</i> Barbados |
|--------------|--|--------------------------------|--|---------------------------|
| FL           | 32.6   | 32.3 (31.4–33.3) 5             | 33.5                                     | 35.3 (34.6–35.9) 7        |
| 3ML          | 30.1   | 29.5 (28.5–30.3) 4             | –  | 32.2 (31.4–32.7) 7        |
| LDF          | 5.6  | 7.0 (6.0–8.0) 4                | 5.9                                      | 6.2–6.5 (2)               |
| LVF          | 4.5  | 5.0 (5.0–6.0) 4                | 3.7                                      | 4.0–5.0 (2)               |
| MAL          | 9.2  | 9.2 (8.9–9.5) 10               | 9.1                                      | 10.4 (10.2–10.6) 7        |
| MAN          | 5.1  | 5.2 (5.1–5.3) 10               | 5.1                                      | 5.9 (5.4–6.0) 7           |
| GLS          | –  | 12.9 (12.5–13.1) 10            | 13.2                                     | 14.3 (14.1–14.4) 7        |
| CCL          | 11.6   | 11.5 (11.1–11.7) 10            | 11.7                                     | 12.8 (12.6–13.1) 7        |
| CBL          | –  | 12.1 (11.8–12.4) 10            | 12.2                                     | 13.5 (13.3–13.7) 7        |
| CIL          | –  | 12.3 (12.0–12.6) 10            | 12.4                                     | 13.8 (13.4–14.0) 7        |
| BAL          | –  | 11.0 (10.8–11.4) 10            | 11.2                                     | 12.5 (12.1–12.7) 7        |
| ZYG          | 8.2  | 7.9 (7.8–8.1) 6                | 8.3                                      | (8.1–8.3) 2               |
| MAB          | 6.7  | 6.5 (6.4–6.7) 10               | 6.7                                      | 7.2 (6.9–7.3) 7           |
| BCB          | 6.2  | 6.0 (5.9–6.2) 10               | 6.2                                      | 6.6 (6.4–6.7) 7           |
| IOB          | 4.2  | 4.2 (4.1–4.3) 10               | 4.0                                      | 4.3 (4.0–4.6) 7           |
| POB          | 3.6  | 3.2 (3.2–3.5) 10               | 3.4                                      | 3.3 (3.3–3.4) 7           |
| BAC          | 3.1  | 3.1 (2.9–3.3) 10               | 3.2                                      | 3.6 (3.6–3.8) 7           |
| BAM          | 5.0  | 5.2 (5.1–5.3) 10               | 5.2                                      | 5.4 (5.4–5.5) 7           |
| MTL          | 4.8  | 4.9 (4.8–5.0) 10               | 4.8                                      | 5.6 (5.4–5.7) 7           |
| M1M3         | 2.7  | 2.8 (2.7–2.8) 10               | 2.7                                      | 3.0 (2.9–3.0) 7           |



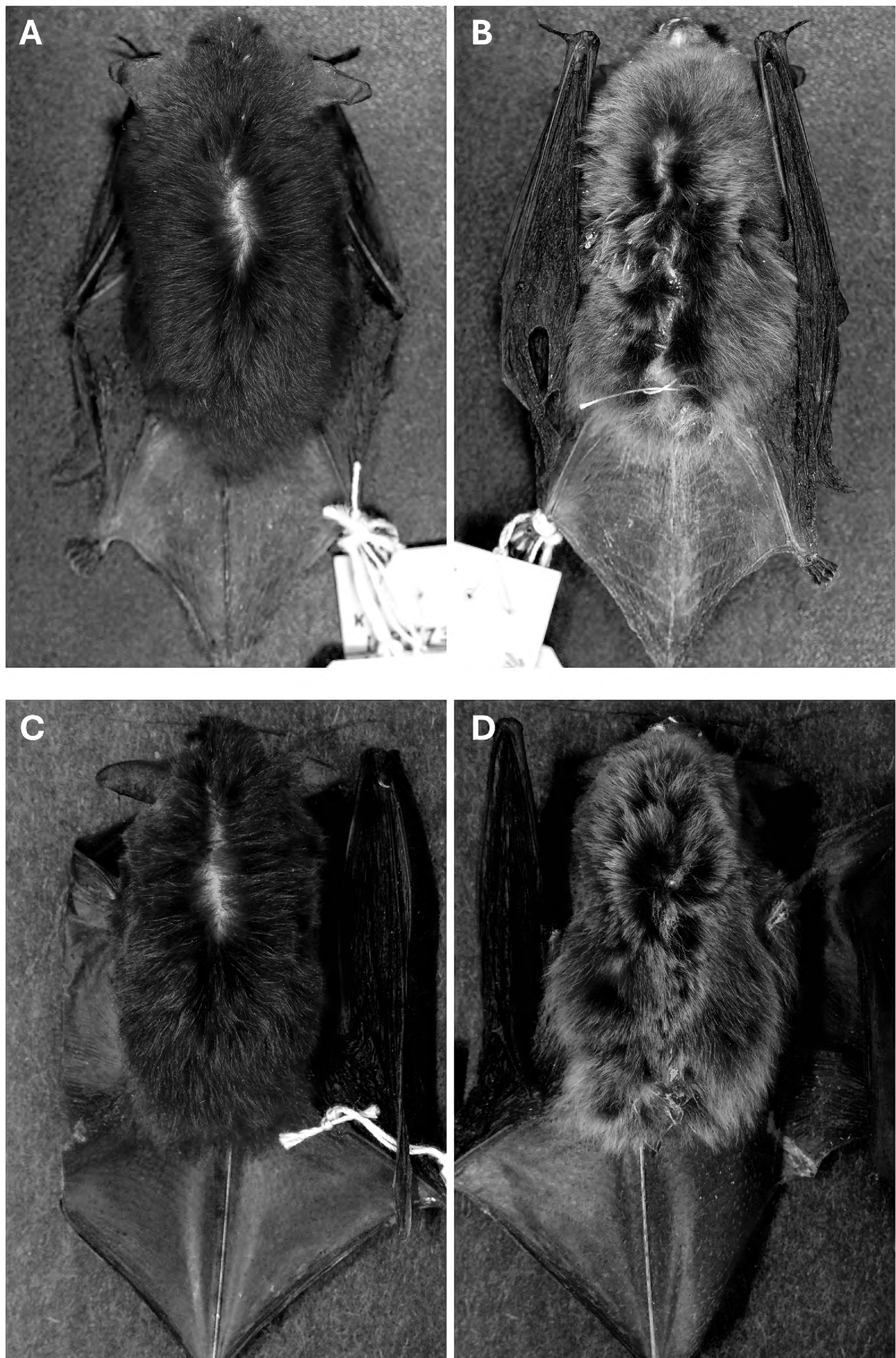


**Figure 3.** Skull profiles of *Myotis attenboroughi* (USNM 252600) from Grenada Island in dorsal, ventral, and lateral views.

The present record of *M. attenboroughi* on the island of Grenada extends the geographic range of this species to two localities in the Caribbean islands (Grenada and Tobago) and one locality in the mainland South America (Suriname; Fig. 7). It is likely that this species has an even wider distribution in northern South America and may oc-

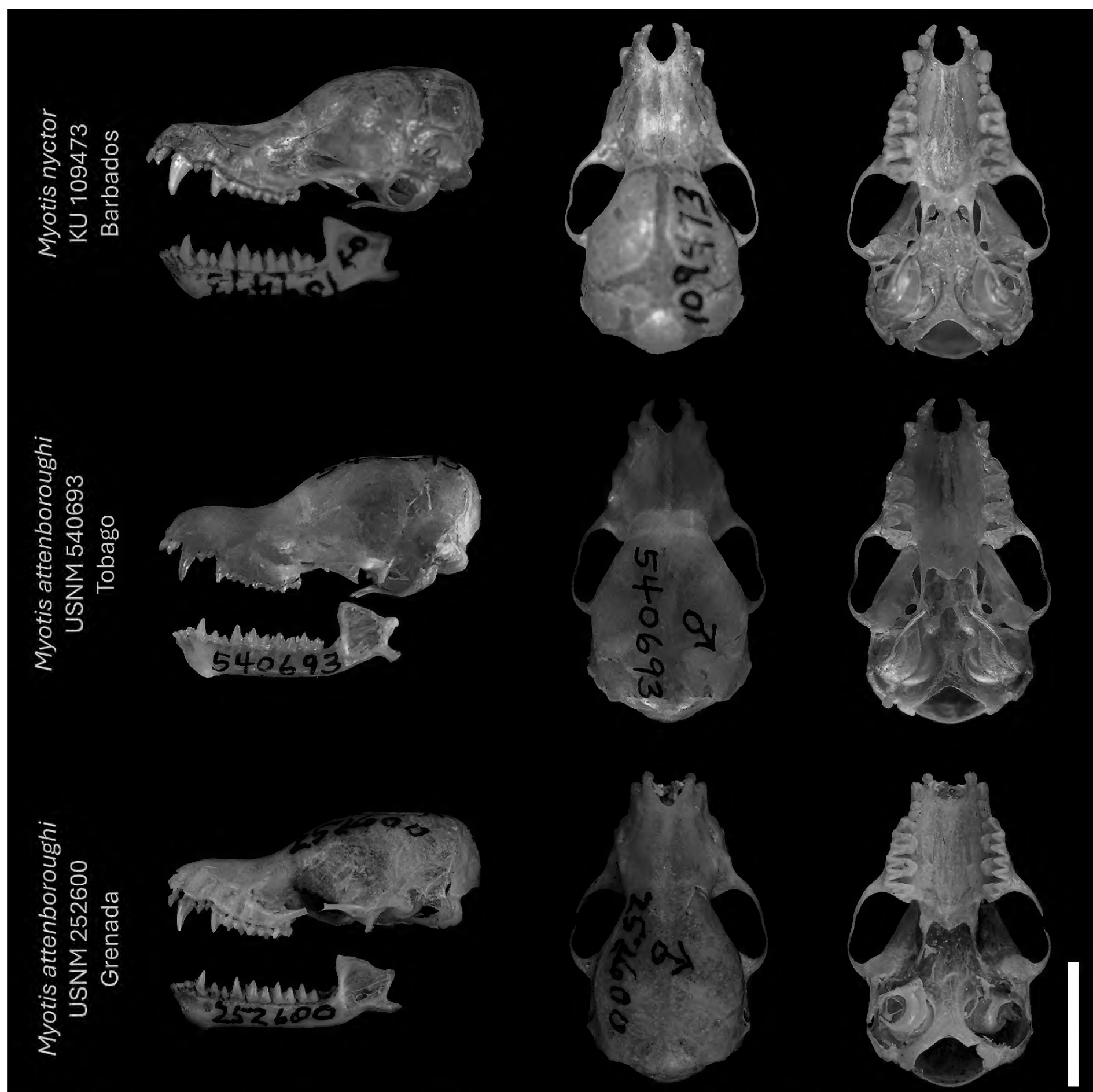
cur in the lowlands along coastal rainforests of the Guiana Shield and northeastern Venezuela, which corroborates the observations made by Genoways et al. (1998).

The specimen CM 83427 morphologically matches with the diagnosis of *M. attenboroughi* (see Moratelli et al. 2017), being virtually identical to USNM 252600 from Grenada.



**Figure 4.** Skins of the paratype of *Myotis nyctor* (KU 109473) from Barbados (A, B) and holotype of *Myotis attenboroughi* (USNM 540693) from Tobago (C, D).





**Figure 5.** Skulls of the paratype of *Myotis nyctor* (KU 109473) from Barbados, holotype of *Myotis attenboroughi* (USNM 540693) from Tobago, and a specimen of *Myotis attenboroughi* (USNM 252600) from Grenada.

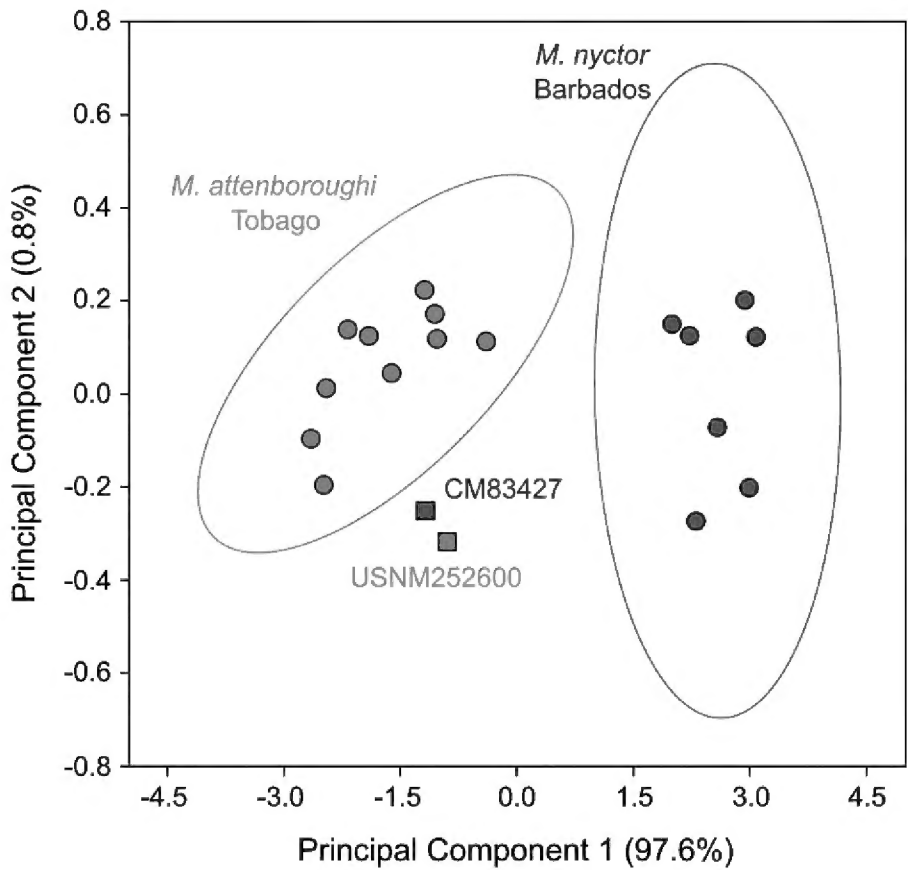
However, our phylogenetic inference recovered this specimen as belonging to the *M. nyctor* clade from Barbados, as previously shown by Larsen et al. (2012), revealing a surprising incongruence between genotype and phenotype. One could argue that the discrepancy between morphological and molecular analyses may be due to issues related with the tissue sampling for DNA analysis and/or with the lab routine for molecular data generation. Although this hypothesis cannot be completely ruled out without the re-sequencing these specimens, at least we can attest to the best practices while working with historical DNA for the individual USNM 252600. Sampling followed rigorous procedures described and tested in McDonough et al. (2018) and Abreu et al. (2020), and data generation (DNA extraction and genomic library preparation) took place in state-of-the-

art facilities with protocols and equipment exclusively used for ancient and historical samples. Moreover, for specimen USNM 252600 we also generated thousands of nuclear ultraconserved elements (data not shown), and these data also corroborate the phylogenetic placement found here for this specimen. Therefore, if data generation issues were ruled out, this unexpected result should be explained in light of the complex evolutionary history of Caribbean *Myotis*.

The Caribbean *Myotis* assemblage originated from multiple overwater dispersals from northern South America to the Lesser Antilles in the Plio-Pleistocene interval (3.2–1.4 mya), which included posterior reverse colonization from the Caribbean to mainland Central and South Americas (Baker and Genoways 1978; Stadelmann et al. 2007; Larsen et al. 2012; Novaes et al. 2021). This

pattern can also be observed in other insectivorous bats (e.g., Dávalos 2005, 2006; Genoways et al. 2005; Pavan et al. 2013). Dispersions may have been mediated by the migration arc formed by the expansion of land area of the Lesser Antilles islands due to sea level retreat during the Quaternary glaciations (Koopman 1958; Genoways

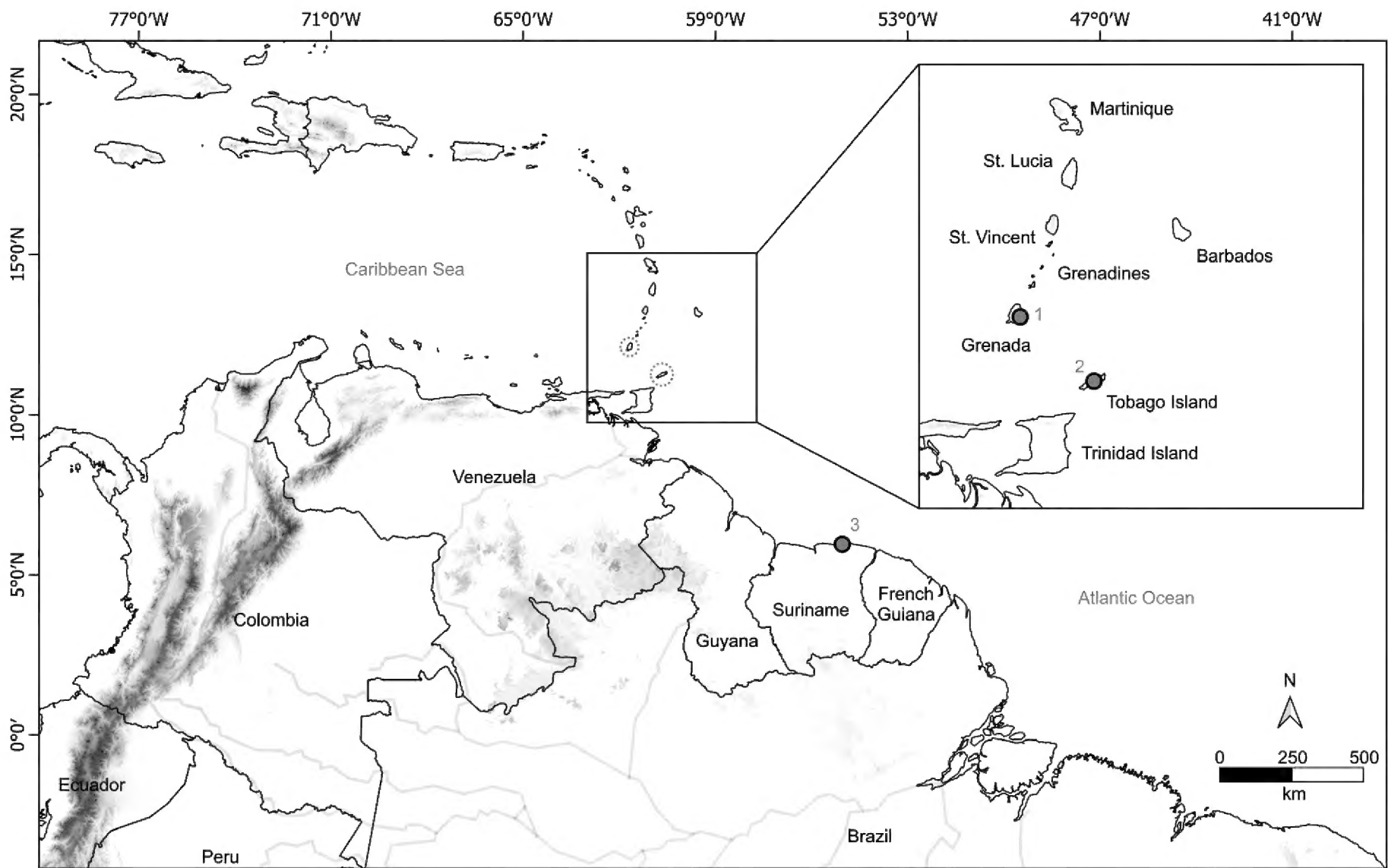
et al. 2010; Dávalos and Russell 2012; Allen et al. 2019; Hoffman et al. 2019). This was especially important for Grenada, Trinidad, Tobago, and northern South America, which allowed a great exchange of species (Koopman 1958; Genoways et al. 2010). Thus, the presence of *M. attenboroughi* in Grenada, Tobago, and Suriname – and probably along a broader area in northern South America – can be explained by the intermittent connections between these islands and the continent during the Plio-Pleistocene glaciations. In fact, some studies consider the fauna of Grenada and Grenadines (united as a single island during



**Figure 6.** Dispersion points of the Principal Component Analysis based on skull measurements *Myotis attenboroughi* from Tobago (red dots), *Myotis nyctor* from Barbados (gray dots), the newly discovered *M. attenboroughi* from Grenada (red square; USNM 252600), and the specimen of *M. cf. nyctor* from Grenada (gray square; CM 83427).

**Table 4.** Vector correlation loadings with original variables of principal components analysis for *Myotis attenboroughi* and *M. nyctor* samples.

| Measurements | PC 1  | PC 2   |
|--------------|-------|--------|
| MAL          | 0.331 | 0.260  |
| MAN          | 0.194 | 0.243  |
| GLS          | 0.369 | -0.298 |
| CCL          | 0.366 | 0.037  |
| CBL          | 0.386 | -0.178 |
| CIL          | 0.408 | -0.063 |
| BAL          | 0.388 | -0.143 |
| MAB          | 0.168 | -0.061 |
| BCB          | 0.123 | -0.163 |
| IOB          | 0.032 | 0.650  |
| POB          | 0.011 | -0.157 |
| BAC          | 0.130 | 0.215  |
| BAM          | 0.124 | 0.317  |
| MTL          | 0.182 | 0.242  |
| M1M3         | 0.064 | 0.202  |



**Figure 7.** Occurrences localities of *Myotis attenboroughi* in Caribbean islands of Grenada (1) and Tobago (2); and mainland South America in Paramaribo, Suriname (3).



the Last Glacial Maximum) as small representation of the South American bat fauna (e.g., Koopman 1958; Genoways et al. 2010; Pavan et al. 2013).

On the other hand, the presence of a Grenadian *Myotis* genotypically closer to *M. nyctor* from Barbados cannot be explained by this scenario. Barbados and Grenada are separated by ca. 240 km in the Tobago Basin, with an ocean depth of more than 2,000 m and no oceanic ridges that may have facilitated the connection of these two populations during glaciation periods (Speed 1981; Humphrey 1997). In addition, Barbados was completely or partially underwater until 1 mya, and perhaps as recent as 700,000 years ago (Speed and Keller 1993; Lovette et al. 1999). So, the arrival of the *M. nyctor* ancestral lineage in Barbados is quite recent and probably resulted from an extreme overwater dispersal event from South America (Larsen et al. 2012a). Therefore, it is possible to assume that other similar events, perhaps driven by wind streams during tropical storms (Hurme et al. 2025), may have facilitated the invasion of *M. nyctor* from Barbados into Grenada. Subsequently, introgression of lineages may have occurred through hybridization, which can explain the discordance between the phenotype (like *M. attenboroughi*) and the genotype (like *M. nyctor*) of the specimen CM 83427 (JN020562) from Grenada. Recent studies reveal a complex evolutionary history of New World *Myotis*, which include several events of historical and modern introgression of lineages, hybridization, incomplete lineage sorting, and gene flow in phenotypically similar non-sister species (Morales and Carstens 2018; Platt et al. 2018; Korstian et al. 2022, 2024).

The hypotheses above are speculative, and new studies are necessary to understand the history of the Caribbean *Myotis* assemblage and the evolutionary processes linked to colonization and diversification. Genome-wide sequences for the Barbados and Grenada specimens should advance our ability to answer these questions since the cytb-only dataset seems to have limited resolution power for the Caribbean clade, restricting data interpretation (Novaes et al. 2021). Furthermore, recent studies have shown discordance between the mitochondrial and nuclear genomes of Neotropical *Myotis*, resulting from the aforementioned evolutionary phenomena (Platt et al. 2018; Korstian et al. 2024). Still, there is robust evidence indicating that the Caribbean is an important diversification center for bats, and that the fauna of the Lesser Antilles was formed from multiple overwater dispersal from northern South America, with reverse colonization of species that invaded the continent after speciation (Hedges et al. 1992; Dávalos 2004, 2007; Pavan et al. 2013; Rojas et al. 2016; Tavares et al. 2018). In this way, the high richness of species, endemism, and presence on the different islands makes *Myotis* an important model for research into biogeography and evolution of the Caribbean.

## Author contributions

RLMN, VCC, and RM conceptualized the study; RLMN, VCC, EFA, DEW, JEM, and RM collected and generated

the data; RLMN and NAB analyzed the data; RLMN and VCC drafted the manuscript; all authors contributed to the theoretical conception of the study and contribute to the final version of the manuscript.

## Data availability

All the data that support the findings of this study are available in the main text. DNA sequences generated in this study have been deposited in NCBI's GenBank. Morphological data matrices for all specimens are available upon request from the corresponding author.

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Appendix 1

**Table A1.** Cytochrome b sequences used in phylogenetic analyses. The information presented for the taxonomic terminals is the result of re-identification of the specimens and does not necessarily coincide with the original identifications provided by the authors and GenBank. Abbreviations for specimen deposit institutions are: Universidad Autónoma Metropolitana-Iztapalapa (UAMI, Ciudad de México, Mexico); Pontificia Universidad Católica del Ecuador (QCAZ, Quito, Ecuador), Carnegie Museum of Natural History (CM, Pittsburg, USA); Field Museum of Natural History (FMNH, Chicago, USA), Museum of Southwestern Biology, University of New Mexico (MSB, Albuquerque, USA), Museum of Vertebrate Zoology, University of California (MVZ, Berkeley, USA), University of Nebraska State Museum (UNSM-ZM, Lincoln, USA); Sam Noble Oklahoma Museum of Natural History (OMNH, Norman, USA); Smithsonian’s National Museum of Natural History (USNM, Washington, DC, USA); Texas Tech University (TTU, Lubbock, USA), Biology Department of Tunghai University (THUMB, Taichung, Taiwan). \*The cytb sequence of *M. attenboroughi* from Grenada (USNM 252600) is available as Suppl. material 1.

| Species                       | #GenBank | Voucher       | Locality                      | Source                          |
|-------------------------------|----------|---------------|-------------------------------|---------------------------------|
| <i>Myotis albescens</i>       | JX130463 | TTU 85088     | Pastaza, Ecuador              | Larsen et al. (2012b)           |
| <i>Myotis albescens</i>       | JX130522 | TTU 85091     | Pastaza, Ecuador              | Larsen et al. (2012b)           |
| <i>Myotis albescens</i>       | AF376839 | FMNH 162543   | Tarija, Bolivia               | Ruedi and Mayer (2001)          |
| <i>Myotis albescens</i>       | JX130503 | TTU 99124     | Boquerón, Paraguay            | Larsen et al. (2012b)           |
| <i>Myotis albescens</i>       | JX130504 | TTU 99818     | Ñeembucú, Paraguay            | Larsen et al. (2012b)           |
| <i>Myotis arescens</i>        | OP270161 | –             | Araucanía, Chile              | Novaes et al. (2022c)           |
| <i>Myotis arescens</i>        | OP270162 | –             | Araucanía, Chile              | Novaes et al. (2022c)           |
| <i>Myotis arescens</i>        | OP270166 | –             | Coquimbo, Chile               | Novaes et al. (2022c)           |
| <i>Myotis arescens</i>        | AM261888 | –             | Santiago, Chile               | Stadelmann et al. (2007)        |
| <i>Myotis attenboroughi</i>   | JN020573 | UNSM-ZM 29470 | St. George Parish, Tobago     | Larsen et al. (2012a)           |
| <i>Myotis attenboroughi</i>   | JN020574 | UNSM-ZM 29483 | St. George Parish, Tobago     | Larsen et al. (2012a)           |
| <i>Myotis attenboroughi</i>   | PQ757917 | USNM 540692   | St. George Parish, Tobago     | Present study                   |
| <i>Myotis attenboroughi</i> * | –        | USNM 252600   | St. David, Grenada Island     | Present study                   |
| <i>Myotis attenboroughi</i>   | JX130505 | CM 77705      | Paramaribo, Suriname          | Larsen et al. (2012b)           |
| <i>Myotis armiensis</i>       | JX130435 | TTU 39146     | Chiriquí, Panama              | Larsen et al. (2012b)           |
| <i>Myotis armiensis</i>       | MW025265 | MSB 262089    | Chiriquí, Panama              | Carrión-Bonilla and Cook (2020) |
| <i>Myotis armiensis</i>       | MW025266 | MSB 262237    | Chiriquí, Panamá              | Carrión-Bonilla and Cook (2020) |
| <i>Myotis armiensis</i>       | MW025267 | MSB 262788    | Chiriquí, Panamá              | Carrión-Bonilla and Cook (2020) |
| <i>Myotis armiensis</i>       | MW025268 | MSB 262085    | Chiriquí, Panamá              | Carrión-Bonilla and Cook (2020) |
| <i>Myotis armiensis</i>       | JX130514 | TTU 85060     | Tungurahua, Ecuador           | Larsen et al. (2012b)           |
| <i>Myotis armiensis</i>       | MW025269 | QCAZ 17245    | Napo, Ecuador                 | Carrión-Bonilla and Cook (2020) |
| <i>Myotis armiensis</i>       | MW025274 | QCAZ 12461    | Zamora Chinchipe, Ecuador     | Carrión-Bonilla and Cook (2020) |
| <i>Myotis armiensis</i>       | MZ345121 | USNM 370890   | Distrito Federal, Venezuela   | Novaes et al. (2022a)           |
| <i>Myotis atacamensis</i>     | OP270158 | –             | Arica, Chile                  | Novaes et al. (2022c)           |
| <i>Myotis atacamensis</i>     | OP270159 | –             | Arica, Chile                  | Novaes et al. (2022c)           |
| <i>Myotis atacamensis</i>     | OP270160 | –             | Arica, Chile                  | Novaes et al. (2022c)           |
| <i>Myotis bakeri</i>          | AM261882 | MVZ 168933    | Olmos, Peru                   | Stadelmann et al. (2007)        |
| <i>Myotis caucensis</i>       | JX130484 | CM 98860      | Huánuco, Peru                 | Larsen et al. (2012b)           |
| <i>Myotis caucensis</i>       | JX130538 | TTU 46346     | Huánuco, Peru                 | Larsen et al. (2012b)           |
| <i>Myotis causensis</i>       | JX130495 | QCAZ 6313     | Pastaza, Ecuador              | Larsen et al. (2012b)           |
| <i>Myotis chiloensis</i>      | OP270163 | –             | Los Lagos, Chile              | Novaes et al. (2022c)           |
| <i>Myotis chiloensis</i>      | OP270164 | –             | Los Lagos, Chile              | Novaes et al. (2022c)           |
| <i>Myotis chiloensis</i>      | OP270165 | –             | Los Lagos, Chile              | Novaes et al. (2022c)           |
| <i>Myotis clydejonesi</i>     | JX130520 | TTU 109227    | Sipaliwini, Suriname          | Larsen et al. (2012b)           |
| <i>Myotis clydejonesi</i>     | JX130453 | CM 98859      | Huánuco, Peru                 | Larsen et al. (2012b)           |
| <i>Myotis diminutus</i>       | JX130447 | TTU 103805    | Loja, Ecuador                 | Larsen et al. (2012b)           |
| <i>Myotis diminutus</i>       | JX130448 | QCAZ 9601     | Esmeraldas, Ecuador           | Larsen et al. (2012b)           |
| <i>Myotis diminutus</i>       | JX130466 | QCAZ 9154     | Esmeraldas, Ecuador           | Larsen et al. (2012b)           |
| <i>Myotis diminutus</i>       | JX130467 | QCAZ 9155     | Esmeraldas, Ecuador           | Larsen et al. (2012b)           |
| <i>Myotis dinellii</i>        | JX130475 | TTU 66489     | Córdoba, Argentina            | Larsen et al. (2012b)           |
| <i>Myotis dinellii</i>        | MT262853 | MG-ZV-M 217   | Zavalla, Argentina            | Caraballo et al. (2020)         |
| <i>Myotis dinellii</i>        | MT262857 | MG-ZV-M 233   | Chanar Laneado, Argentina     | Caraballo et al. (2020)         |
| <i>Myotis dominicensis</i>    | JN020555 | TTU 31507     | St. Joseph’s Parish, Dominica | Larsen et al. (2012a)           |
| <i>Myotis dominicensis</i>    | JN020556 | TTU 31508     | St. Joseph’s Parish, Dominica | Larsen et al. (2012a)           |
| <i>Myotis dominicensis</i>    | AF376848 | –             | St. Joseph’s Parish, Dominica | Ruedi and Mayer (2001)          |
| <i>Myotis elegans</i>         | JX130479 | TTU 84380     | Atlantida, Honduras           | Larsen et al. (2012b)           |
| <i>Myotis elegans</i>         | JX130480 | TTU 84138     | Atlantida, Honduras           | Larsen et al. (2012b)           |
| <i>Myotis extremus</i>        | AF376852 | –             | Yucatán, Mexico               | Ruedi and Mayer (2001)          |



| Species                         | #GenBank | Voucher       | Locality                      | Source                          |
|---------------------------------|----------|---------------|-------------------------------|---------------------------------|
| <i>Myotis extremus</i>          | JX130449 | TTU 47514     | Yucatán, Mexico               | Larsen et al. (2012b)           |
| <i>Myotis extremus</i>          | JX130525 | –             | Yucatán, Mexico               | Larsen et al. (2012b)           |
| <i>Myotis extremus</i>          | JX130489 | CM 55764      | Veracruz, Mexico              | Larsen et al. (2012b)           |
| <i>Myotis extremus</i>          | MF143477 | –             | Veracruz, Mexico              | Platt et al. (2018)             |
| <i>Myotis extremus</i>          | MW025270 | MVZ 226977    | Alta Verapaz, Guatemala       | Carrión-Bonilla and Cook (2020) |
| <i>Myotis fortidens</i>         | JX130437 | –             | Michoacán, Mexico             | Larsen et al. (2012b)           |
| <i>Myotis fortidens</i>         | JX130439 | –             | Michoacán, Mexico             | Larsen et al. (2012b)           |
| <i>Myotis fortidens</i>         | KC747690 | LACM 73713    | Guerrero, Mexico              | Patrick and Stevens (2014)      |
| <i>Myotis keaysi</i>            | JX130516 | QCAZ 11380    | Chimborazo, Ecuador           | Larsen et al. (2012b)           |
| <i>Myotis keaysi</i>            | JX130517 | QCAZ 11383    | Chimborazo, Ecuador           | Larsen et al. (2012b)           |
| <i>Myotis keaysi</i>            | MW025273 | MSB 70381     | Cochabamba, Bolivia           | Carrión-Bonilla and Cook (2020) |
| <i>Myotis larensis</i>          | JN020569 | TTU 48161     | Guárico, Venezuela            | Larsen et al. (2012b)           |
| <i>Myotis larensis</i>          | JX130529 | TTU 48162     | Guárico, Venezuela            | Larsen et al. (2012b)           |
| <i>Myotis larensis</i>          | JX130535 | CM 78645      | Guárico, Venezuela            | Larsen et al. (2012b)           |
| <i>Myotis lavalii</i>           | AF376864 | MVZ 185681    | Paraíba, Brazil               | Ruedi and Mayer (2001)          |
| <i>Myotis martiniquensis</i>    | AM262332 | –             | Martinique Island             | Stadelmann et al. (2007)        |
| <i>Myotis martiniquensis</i>    | JN020557 | MNHN 2005-895 | Grand'Rivière, Martinique     | Larsen et al. (2012a)           |
| <i>Myotis martiniquensis</i>    | JN020558 | MNHN 2005-896 | Le Morne Rouge, Martinique    | Larsen et al. (2012a)           |
| <i>Myotis midastactus</i>       | MW323450 | USNM 584502   | Santa Cruz, Bolivia           | Novaes et al. (2022a)           |
| <i>Myotis moratellii</i>        | JX130572 | QCAZ 9179     | El Oro, Ecuador               | Larsen et al. (2012b)           |
| <i>Myotis moratellii</i>        | MZ345120 | USNM 513482   | Los Ríos, Ecuador             | Novaes et al. (2022a)           |
| <i>Myotis nesopolus</i>         | JN020575 | –             | Bonaire, Netherlands Antilles | Larsen et al. (2012a)           |
| <i>Myotis nesopolus</i>         | JN020576 | –             | Bonaire, Netherlands Antilles | Larsen et al. (2012a)           |
| <i>Myotis nesopolus</i>         | JN020577 | –             | Bonaire, Netherlands Antilles | Larsen et al. (2012a)           |
| <i>Myotis nigricans</i>         | OR187561 | FMA 630       | Rio de Janeiro, Brazil        | Novaes et al. (2024)            |
| <i>Myotis nigricans</i>         | OR187562 | FMA 957       | Rio de Janeiro, Brazil        | Novaes et al. (2024)            |
| <i>Myotis nigricans</i>         | PP584498 | FMA 1525      | Rio de Janeiro, Brazil        | Novaes et al. (2024)            |
| <i>Myotis nigricans</i>         | PP584499 | FMA 1534      | Rio de Janeiro, Brazil        | Novaes et al. (2024)            |
| <i>Myotis</i> cf. <i>nyctor</i> | JN020562 | CM 83427      | St. David Parish, Grenada     | Larsen et al. (2012a)           |
| <i>Myotis nyctor</i>            | JN020563 | TTU 109225    | St. Thomas Parish, Barbados   | Larsen et al. (2012a)           |
| <i>Myotis nyctor</i>            | JN020564 | TTU 109226    | St. Thomas Parish, Barbados   | Larsen et al. (2012a)           |
| <i>Myotis nyctor</i>            | JN020565 | TTU 109229    | St. Thomas Parish, Barbados   | Larsen et al. (2012a)           |
| <i>Myotis nyctor</i>            | JN020566 | TTU 109224    | St. Thomas Parish, Barbados   | Larsen et al. (2012a)           |
| <i>Myotis nyctor</i>            | JN020567 | TTU 109230    | St. Thomas Parish, Barbados   | Larsen et al. (2012a)           |
| <i>Myotis oxyotus</i>           | JX130509 | –             | Loja, Ecuador                 | Larsen et al. (2012b)           |
| <i>Myotis oxyotus</i>           | JX130585 | –             | Loja, Ecuador                 | Larsen et al. (2012b)           |
| <i>Myotis oxyotus</i>           | MW089499 | QCAZ 11739    | Imbabura, Ecuador             | Carrión-Bonilla et al. (2024)   |
| <i>Myotis pilosatibialis</i>    | JX130526 | TTU 35360     | San Luis Potosí, Mexico       | Larsen et al. (2012b)           |
| <i>Myotis pilosatibialis</i>    | JX130518 | TTU 35631     | San Luis Potosí, Mexico       | Larsen et al. (2012b)           |
| <i>Myotis pilosatibialis</i>    | MW025271 | MVZ 226976    | Alta Verapaz, Guatemala       | Carrión-Bonilla and Cook (2020) |
| <i>Myotis pilosatibialis</i>    | MW025272 | MVZ 226973    | El Quiche, Guatemala          | Carrión-Bonilla and Cook (2020) |
| <i>Myotis pilosatibialis</i>    | MW025275 | MVZ 224798    | Quezaltenango, Guatemala      | Carrión-Bonilla and Cook (2020) |
| <i>Myotis pilosatibialis</i>    | JX130519 | TTU 60981     | Santa Ana, El Salvador        | Larsen et al. (2012b)           |
| <i>Myotis riparius</i>          | JX130492 | TTU 102883    | Esmeraldas, Ecuador           | Larsen et al. (2012b)           |
| <i>Myotis riparius</i>          | JX130473 | CM 68443      | Para, Suriname                | Larsen et al. (2012b)           |
| <i>Myotis riparius</i>          | JX130474 | CM 78659      | Bolívar, Venezuela            | Larsen et al. (2012b)           |
| <i>Myotis riparius</i>          | MW089495 | MSB 70383     | Cochabamba, Bolivia           | Carrión-Bonilla et al. (2024)   |
| <i>Myotis riparius</i>          | JX130485 | TTU 99645     | Paraguari, Paraguay           | Larsen et al. (2012b)           |
| <i>Myotis riparius</i>          | MW089493 | OMNH 36220    | Tucumán, Argentina            | Carrión-Bonilla et al. (2024)   |
| <i>Myotis ruber</i>             | AF376867 | MVZ 185999    | São Paulo, Brazil             | Ruedi and Mayer (2001)          |
| <i>Myotis simus</i>             | JX130481 | TTU 46348     | Huánuco, Peru                 | Larsen et al. (2012b)           |
| <i>Myotis velifer</i>           | AF376870 | MVZ 146766    | Sonora, Mexico                | Ruedi and Mayer (2001)          |
| <i>Myotis velifer</i>           | JX130438 | UAMI 15306    | Michoacán, Mexico             | Larsen et al. (2012b)           |
| <i>Myotis velifer</i>           | JX130589 | UAMI 15305    | Michoacán, Mexico             | Larsen et al. (2012b)           |
| <i>Myotis yumanensis</i>        | AF376875 | MVZ 15585     | California, USA               | Stadelmann et al. (2007)        |
| <i>Myotis</i> sp. 1             | JN020570 | CM 63933      | Nickerie, Suriname            | Larsen et al. (2012a)           |
| <i>Myotis</i> sp. 1             | JN020571 | CM 69053      | Para, Suriname                | Larsen et al. (2012a)           |
| <i>Myotis</i> sp. 1             | JN020572 | CM 77699      | Para, Suriname                | Larsen et al. (2012a)           |
| <i>Myotis</i> sp. 1             | JX130476 | CM 77692      | Marowjine, Suriname           | Larsen et al. (2012b)           |
| <i>Myotis</i> sp. 1             | JX130534 | CM 77694      | Nickerie, Suriname            | Larsen et al. (2012b)           |
| <i>Myotis</i> sp. 1             | JX130536 | CM 77700      | Para, Suriname                | Larsen et al. (2012b)           |

| Species                        | #GenBank | Voucher     | Locality                   | Source                   |
|--------------------------------|----------|-------------|----------------------------|--------------------------|
| <i>Myotis</i> sp. 2            | AF376865 | FMNH 129208 | Lima, Peru                 | Ruedi and Mayer (2001)   |
| <i>Myotis</i> sp. 3            | MT262866 | MFA-ZV 1425 | Esperanza, Argentina       | Caraballo et al. (2020)  |
| <i>Myotis</i> sp. 3            | JX130450 | TTU 34952   | Puerto Linares, Bolívia    | Larsen et al. (2012b)    |
| <i>Myotis</i> sp. 3            | JX130528 | TTU 34953   | Puerto Linares, Bolívia    | Larsen et al. (2012b)    |
| <i>Myotis</i> sp. 3            | PP584500 | UFMT 4946   | Mato Grosso, Brazil        | Novaes et al. (in press) |
| <i>Myotis</i> sp. 3            | PP584501 | MZUFV 5180  | Mato Grosso, Brazil        | Novaes et al. (in press) |
| <i>Myotis</i> sp. 3            | JX130498 | TTU 99046   | Alto Paraguai, Paraguay    | Larsen et al. (2012b)    |
| <i>Myotis</i> sp. 3            | JX130455 | TTU 95992   | Alto Paraguai, Paraguay    | Larsen et al. (2012b)    |
| <i>Myotis</i> sp. 3            | JX130540 | TTU 99151   | Boquerón, Paraguay         | Larsen et al. (2012b)    |
| <i>Myotis</i> sp. 3            | JX130539 | TTU 99516   | Concepción, Paraguay       | Larsen et al. (2012b)    |
| <i>Myotis</i> sp. 3            | JX130499 | TTU 99802   | Ñeembucu, Paraguay         | Larsen et al. (2012b)    |
| <i>Myotis</i> sp. 3            | JX130496 | TTU 99743   | Presidente Hayes, Paraguay | Larsen et al. (2012b)    |
| Outgroups                      |          |             |                            |                          |
| <i>Myotis emarginatus</i>      | MK799667 | FMNH 178892 | Ajlun, Jordan              | Patterson et al. (2019)  |
| <i>Submyotodon latirostris</i> | KP187906 | THUMB 30036 | Heping, Taiwan             | Ruedi et al. (2015)      |
| <i>Kerivoula papillosa</i>     | MG194454 | FMNH 205343 | Luzon I, Philippine Island | Sedlock et al. (2020)    |

Appendix 2

Specimens examined in morphological comparisons. These vouchers consist of fluid preserved specimens, stuffed skins, and skulls deposited in the American Museum of Natural History (AMNH, New York, United States); Carnegie Museum of Natural History (CM, Pittsburgh, United States); National Museum of Natural History, Smithsonian Institution (USNM, Washington, D.C., United States); Natural History Museum, University of Kansas (KU, Lawrence, United States).

*Myotis attenboroughi* (N = 14): Trinidad and Tobago: Tobago Island, Charlottesville, 1 km N of Pirate’s Bay, Saint John Parish (USNM 540693 [holotype], 540692 [paratype]); Tobago Island, St. Mary Parish, Hillsborough Reservoir (USNM 538064, 538065, 538066, 538067, 538068, 538069, 540619, 540620, 540621, 540694, 540695 [paratypes]). Grenada: St. David (USNM 252600).

*Myotis nyctor* (N = 8): Barbados: St. Thomas Parish, Cole’s cave (KU 151761, 151762, 151763, 151764, 151765, 151766, 109473 [paratype]); St. Thomas, Near Cole’s cave (AMNH 213926).

*Myotis* cf. *nyctor* (N = 1): Grenada, St. David (CM 83427).

Supplementary material 1

Supplementary data

Authors: Roberto Leonan M. Novaes, Vinícius C. Cláudio, Natasha A. Bertocchi, Edson F. Abreu, Don E. Wilson, Jesús E. Maldonado, Ricardo Moratelli

Data type: fasta

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